RANCIDITY IN EVISCERATED POULTRY

by

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of Agriculture and Applied Science, 1945

A THESIS

submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1948

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INTRODUCTION

During World War II great loss of food was suffered through non-acceptance of many stored food products by the soldier consumer. This brought about increased research efforts for improvements in procedures of preserving foods. The method of preserving poultry by freezing has been very satisfactory and popular within recent years, but prolonged storage periods and poor storage conditions often times decreased the quality of the meat.

Schreiber, Vail, Conrad and Payne (17) and Wagoner, Vail and Conrad (18) found that oxidative rancidity was the main cause of flavor deterioration in frozen poultry. According to Barnes, Lundberg, Hanson and Burr (1), "There are two basic factors of importance governing the resistance of natural fats to rancidification. These are (1) the composition of the component glycerides and (2) the amount and nature of existing natural antioxidants." Cruickshank (5) and Hilditch, Jones and Rhead (9) have shown that the degree of unsaturation of the dietary fat had a great influence on the character of the depot fats in poultry. It is agreed generally that the more unsaturated dietary fats produce unstable body fats. Cruickshank (5) showed that the ingestion of the un-

saturated fatty acids in hempseed produced a "marked and rapid increase in unsaturation" of the mixed fatty acids of the depot fats in fowl. The work of Schreiber, et al. (17) has shown that the feeding of alfalfa or fish oils decreased the storage life of frozen poultry.

To date, efforts to find natural antioxidants have been unsuccessful. Barnes, et al. (1) discovered tocopherols, above the normal dietary amounts, did not increase the stability of rat fat. Overman (15) found that ascorbic acid and hydroquinone were also unsatisfactory. The results of Kummerow, Hite and Kloxin (11) seemed to indicate that it may be possible to stabilize tissue fats by feeding ethanolamine or choline. These data did not indicate the exact function of the supplements. However, the results suggested that the feeding of ethanolamine or choline may have (1) changed the proportion of various fats in the skin tissue or (2) affected the fat metabolism.

Another approach to the problem of rancidity in frozen poultry is the study of the composition of the extracted fats in relation to their stability to oxidation. Until recently, such a study has been limited by the methods available for determining the components of the fats. With the development of spectrophotometric methods by Mitchell, Kraybill and Zscheile (13), Bradley and Richardson (4) and Beadle and Kraybill (2) it was possible to determine the fatty acid composition of poultry fats. Holman and Elmer (10) found

that, "The increase in the number of double bonds in a fatty acid by one increases the rate of oxidation of the fatty acid or its esters by at least a factor or two." The fatty acid composition of extracted poultry fat might therefore be expected to reflect the stability to oxidative rancidity during cold storage.

In the present study of rancidity in eviscerated poultry, turkeys were used instead of chickens. Observations have shown that stored turkeys became rancid faster than chickens. This difference may be due mainly to the use of compounds with more unsaturated fatty acids in turkey rations. In the present work dietary fats of varying degree of unsaturation were added to the basal ration. Hydrogenated fat, linseed oil and the extract of alfalfa leaf meal were used as dietary sources of fat. Groups which had been fed a diet containing these fats plus ethanolamine and choline were compared with birds which had received the fat-enriched basal rations to which no supplements had been added. One group was retained as a control group and was fed only the basal ration. Ethanolamine and choline also were added to the diets of turkeys raised on a regular poultry ration. The effects of these dietary constituents on the composition of the body fats and on the storage life of the frozen eviscerated poultry were studied.

EXPERIMENTAL

One-hundred and seventy-five Broad-breasted Bronze poults of the Kansas State College strain were used for this experiment. The poults were from the same hatch and were started on the two basal rations shown in Tables 1 and 2 when they were one day old. One-hundred and twenty-five poults were fed ration #1 and the remaining 50 were fed ration #2. After eight weeks, the 125 poults on ration #1 were divided into nine groups and fed different supplements. Each group was kept in a converted brooder house and separated from the others by a wire enclosure. A fenced gravel runway outside each enclosure permitted ready access to sunlight and fresh air for all the turkeys.

The 50 poults on ration #2 were divided into three groups. A control was fed the basal ration, a second received choline hydrochloride and the third received neutralized ethanolamine. These birds were allowed to run in a wire enclosed grass range simulating the usual farm practice. The various dietary supplements fed to the turkeys were incorporated in the rations, Table 3. The birds were weighed at 8, 12 and 16 weeks of age, Table 4. The turkeys in groups 1 to 9, which received basal ration #1, were killed at 22 weeks of age and dressed under standardized conditions, according to the method of Wagoner, Vail and Conrad (18,19,20). Those on basal ration #2 were killed at 28 weeks of age. Five

birds from each group were wrapped in M.S.A.T. cellophane[1], tied and sealed with scotch tape, and placed in cold storage at -13°C.

The remaining birds in each group were analyzed immediately to determine the influence of the dietary fats and supplements on fat metabolism. The turkeys were skinned and a representative sample used for extraction of fats. The gizzards of all the birds in a group were pooled and extracted. The livers of all turkeys in a group were also combined. The fats of the skin, gizzard and liver were extracted according to a modification of the Bloor method (3). These fat extracts were used to determine the fat content, induction period, iodine value, fatty acid composition, phospholipid content, percentage of phosphorus and percentage of choline as described under experimental procedures.

One stored turkey from each group was analyzed after 4, 9, and 12 months of storage. The skin from the breast and one leg was removed from each turkey and extracted with ethylene chloride as prescribed by Schreiber, et al. (17). The extracted fat was analyzed for its free fatty acid content, peroxide value and aldehyde value. One unskinned leg from each turkey was used for organoleptic

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[1] Courtesy of Du Pont Co., Wilmington, Delaware.

tests. These methods are outlined under experimental procedures.

EXPERIMENTAL PROCEDURES

Extraction of Fat from Tissue with Acetone-Alcohol-Skellysolve F

The skin, gizzard and liver tissues were weighed and minced into small pieces. The skin tissues were cut with scissors and the gizzard and liver tissues were minced by a Waring blender. Approximately 100 g of tissue was placed in a 2-liter Erlenmeyer flask and enough acetone added to cover it. This mixture was refluxed on a steam bath for one hour. The acetone was removed by filtering through a Buchner funnel. The acetone extraction was repeated with fresh acetone and the two acetone extracts were combined and saved. By the same procedure, the tissue residue left after the acetone extraction was refluxed twice with 95 percent ethyl alcohol and then twice with Skellysolve F.

The combined alcohol extracts which contained most of the phospholipids were dried by shaking with anhydrous sodium sulfate and then filtered into a weighed round-bottom flask and the alcohol removed under vacuum.

The acetone and Skellysolve F extracts were combined in a separatory funnel and shaken. The lower layer was drawn off and washed several times with fresh Skellysolve F. The final Skellysolve F washing was clear and only

faintly yellow. The Skellysolve F extracts were combined and washed three times with an equivalent amount of distilled water. The washings were done carefully as emulsions were easily formed and were difficult to break. The Skellysolve F extracts were dried by filtering through anhydrous sodium sulfate.

The dried Skellysolve F extract was then poured into the weighed round-bottom flask which contained the alcohol-soluble extracts. The solvent was removed under vacuum. The extracted fats were cooled, weighed, and taken up and diluted to a volume of 250 ml with Skellysolve F. The fat extracts were stored at -13°C. when not in use. Aliquots of this solution were used for all other tests. The percentage of fat was determined by accurately measuring 3 or 5 ml of the fat extract into a clean, weighed iodine flask. The solvent was removed under vacuum. Upon cooling in a vacuum desiccator the flask was weighed again. The weight of fat present in the aliquot was used to calculate the weight of the total fat extracted. The percentage of fat in the tissue was calculated by the following equation:

$$\text{Percentage crude fat extract} = \frac{\text{weight extract} \times 100}{\text{weight tissue}}$$

Iodine Value(24)

An aliquot containing approximately 0.1 g of fat was carefully pipetted into a glass-stoppered iodine flask. Most of the solvent was evaporated and 5 ml of chloroform

and exactly 15 ml of Wijs solution added. The flasks were stoppered and placed in the dark. At the end of one hour, 10 ml of 15 percent potassium iodide solution were added and mixed thoroughly. The glass stopper and the sides of the flask were washed with 10 ml of distilled water. The sample was then titrated with N/10 thiosulfate solution to a faint yellow. A few drops of starch solution were added and the mixture shaken well to free all the iodine, and titrated to a colorless end point. Two blank determinations were run along with the unknowns.

Wijs solution-Thirteen g of iodine was dissolved in a liter of glacial acetic acid. The solution was heated until all the iodine crystals were dissolved. Chlorine gas was bubbled into the solution until it became light brown in color. The titration of the Wijs solution should be double the titration of the original iodine solution when titrated with N/10 thiosulfate solution.

Potassium iodide solution-Fifteen g of potassium iodide was dissolved in 85 ml of distilled water.

N/10 thiosulfate solution-Twenty-four and eight tenths g of sodium thiosulfate was dissolved in one liter of distilled water and the exact normality determined by the following procedure:

Exactly 10 ml of N/10 potassium dichromate was measured into an iodine flask and 5 ml of concentrated hydrochloric acid added. Ten ml of a 15 percent potassium iodide solution

was added and the mixture was immediately titrated to a greenish color with the thiosulfate solution. Starch indicator was then added and titration continued slowly with occasional shaking until the solution suddenly turned to a clear, bright green.

The formulas used in calculating iodine values were:

$$\text{Normality factor} = \frac{126.9 \times \text{normality of dichromate soln.}}{\text{ml thiosulfate used}}$$

$$\text{Iodine value} = \frac{\text{normality factor} \times (\text{blank titration} - \text{sample})}{\text{sample weight}}$$

Spectrophotometric Analysis(2,4,13)

An aliquot containing 0.1 g of fat was pipetted into a marked and weighed ignition tube. The exact weight of the fat was determined by removing the solvent in a vacuum oven and reweighing the ignition tubes. Two blanks were determined along with unknowns. Exactly 4 ml of alkaline ethylene glycol was added to the ignition tubes. The tubes were stoppered with glass stoppers and placed in a wire basket. The basket was then placed in an oil bath and kept in it at 180°C. ($\pm$2°) for 30 minutes. Before the introduction of the basket, the oil bath had a temperature of about 200° to 210°C. so that the cold basket did not cool the oil below 180°C.

After 30 minutes of heating, the basket was removed from the bath. The contents of the tubes were transferred quantitatively with absolute ethyl alcohol into 100-ml

volumetric flasks. The ignition tubes were washed thoroughly with small portions of absolute alcohol. After the volumetric flasks were made up to volume and mixed well, they were allowed to stand in a cold room at about 10°C. for 5 to 6 hours or overnight. This procedure allowed the silica from the corrosive action of potassium hydroxide on the glass to precipitate.

When warmed to room temperature, the samples were filtered and the first 15 to 20 ml which might contain some foreign substances from the filter paper was discarded. A 10-ml aliquot from each sample was transferred by means of a pipette to a 250-ml volumetric flask. When made up to volume with absolute alcohol and mixed well, the samples were read on a Beckman spectrophotometer at wave lengths 2320, 2620, 2680, 2740, 3100, 3160 and 3220 Å against a blank which had been diluted in the same manner. Other dilutions could be used but the readings must be between 0.2 and 0.3 current density. The readings were recorded along with the dilution.

Alkaline ethylene glycol-Fifteen g of potassium hydroxide was ground and dissolved in 180 ml of ethylene glycol.

Absolute ethyl alcohol-The absolute alcohol could be reused after distilling with a small amount of anhydrous calcium chloride and zinc dust.

The formulas used for calculating the fatty acid composition were:

$$K_2 = \frac{k_{2320}}{\text{wt. sample per liter}} + 0.04$$

$$K_3 = \frac{4.1}{\text{wt. sample per liter}} \left(k_{2680} - \frac{k_{2620} + k_{2740}}{2}\right)$$

$$K_4 = \frac{2.5}{\text{wt. sample per liter}} \left(k_{3160} - \frac{k_{3100} + k_{3220}}{2}\right)$$

where K_{2320}, K_{2620}, etc. designated the spectrophotometric readings of the fat sample at that particular wave length.

Percentage linoleic acid = $1.125K_2 - 1.27K_3 + 0.04$

Percentage linolenic acid = $1.87K_3 - 4.43K_4$

Percentage arachidonic acid = $4.43K_4$

Percentage oleic acid = iodine value x 100 - (181.5 x % linoleic acid - 273.5 x % linolenic acid - 333.5 x % arachidonic acid)/90

Percentage saturated acids = 100 -(% linoleic acid + % linolenic acid + % arachidonic acid + % oleic acid)

Induction Period (8)

Aliquots of extract sufficient to give one g of fat were measured into 24/40 S-joint iodine flasks. The flasks were then placed in a vacuum oven for one hour to remove the solvent. Tank oxygen instead of air was used to bring the vacuum chamber to atmospheric pressure. The flasks were removed from the oven and stoppered with ground-glass stoppers. The actual induction periods were run at 70°C. in a ther-

monstatically controlled water bath, Fig. 1. The flasks were immersed to a depth of ½-inch and held in position by a wooden rack resting on top of the bath. As each flask was placed in the bath the stopper was replaced by a 24/40 S-joint manometer. The manometers were Y shaped. One branch of the Y was ordinary glass tubing and the other branch was capillary tubing. Both branches of the Y, as well as the curved portion of the stem were filled with mercury. Another small glass tube carried a stop-cock projected vertically from the ground-glass fitting. This stop-cock was left open for 15 minutes after placing the flasks in the bath to allow the pressure in the flask to come to equilibrium with atmospheric pressure. While equilibrium conditions were being reached, the manometers were connected with the electromagnetic recorder by means of insolated copper wires. All the flasks were connected to a common ground by means of wires immersed deep in the mercury of the capillary branch of the Y. The other branch of the Y contained a piece of copper wire one cm in length so adjusted that a pressure fall of 10 mm of mercury in the flask caused the circuit through the manometer to break. Each of these wires was connected to a clock mechanism and recorder. An ink writing pen recorded each circuit on a roll of paper once every hour as long as the circuit was unbroken. A pressure fall of 10 mm was taken as the induction period and in this manner

it could be measured to the nearest hour. A careful check on the atmospheric pressure changes was required during a run. A large rise in atmospheric pressure would cause the induction period to be shortened and a fall in atmospheric pressure would cause the induction period to be long.

Phospholipid Precipitation

An aliquot of the total fat was concentrated by evaporating on a steam bath until it was freed from solvent. The fat extract was then poured slowly into a 250-ml centrifuge bottle containing 200 ml cold acetone. The mixture was stirred vigorously with a stirring rod during the addition of the fat extract, then centrifuged until the acetone was clear and the phospholipids were completely precipitated. The acetone fraction was decanted into weighed beakers, the acetone evaporated on a steam bath and freed from solvent in a vacuum oven. The neutral fats were cooled and weighed in order to determine the percentage of neutral fat. The fat was then taken up in Skellysolve F to a suitable volume.

The precipitated phospholipids in the centrifuge bottle were taken up in Skellysolve F to a suitable volume. Aliquots of this solution were used for all tests.

Phosphorus Determination (7,12)

An aliquot containing 0.10 to 0.15 g of fat was pipetted accurately into phosphorus ignition tubes. Two blanks were

run along with the unknowns. A glass wool mat, one inch in diameter, was added to each tube to prevent bouncing. The glass wool had been washed with concentrated sulfuric acid and then repeatedly with distilled water. Seven-tenths ml of concentrated sulfuric acid was pipetted into each tube and the tubes placed on a heating apparatus overnight. Then one half ml of a 30 percent solution of hydrogen peroxide was added to each tube. Caution was exercised to add the peroxide directly onto the sample at the bottom of the tube instead of allowing the peroxide to slide down the tube wall. The contents were shaken and carefully boiled over a free flame to allow the peroxide to evaporate. The tube was then cooled and the peroxide treatment was repeated until the contents of the tube became white and remained that way on further heating. The tubes were cooled to room temperature. Two ml of distilled water and 2 drops of phenolphthalein were added to each tube. By means of a pipette or burette, enough N/10 potassium hydroxide solution was added to give a red coloration to the indicator. Then N/10 sulfuric acid was added drop by drop until the red color just disappeared.

The contents were then filtered quantitatively into 50-ml volumetric flasks through coarse filter paper. The glass wool mat was pulled out onto the moistened filter paper with a glass rod. The ignition tube, the glass rod and the wool mat were then washed carefully with numerous

portions of hot distilled water until the sample was diluted to volume. After shaking, 5 ml from each flask was transferred by means of a pipette to a 25-ml volumetric flask. Then to each of the 25-ml flasks, 0.5 ml of concentrated sulfuric acid, 4 ml of molybdate reagent and 2 ml of sulfonic acid solution were added. The samples were shaken between additions and diluted to volume with distilled water. The samples were allowed to stand for two hours to insure complete color development. The color intensity was read on an Evelyn colorimeter against a blank which was set at 100 percent transmission.

Five standards were run with each test. They were made by adding 0.2, 0.4, 0.6, 0.8 and 1.0 mg of phosphorus from a standard solution into five different 25-ml volumetric flasks. Then 0.5 ml of concentrated sulfuric acid, 4 ml of molybdate reagent and 2 ml of sulfonic acid solution were added. The samples were shaken between each addition. The contents were made up to volume with distilled water, shaken well and read with the samples.

Standard phosphorus solution-Exactly 0.4394 g of dry monopotassium phosphate was dissolved in one liter of water. A few drops of chloroform were added to prevent formation of mold. Each milliliter of this phosphate solution then contained 0.1 mg of phosphorus. Standard solutions were made from this stock solution by further dilution.

Molybdate reagent-Fifteen g of ammonium molybdate was dissolved in 200 ml of distilled water. Two-hundred ml of N/10 sulfuric acid was poured slowly into 400 ml of distilled water. When cool, the sulfuric acid solution was poured into the 200 ml of molybdate solution. The reagent was stored in the dark.

Sulfonic acid solution-Fofteen g of anhydrous sodium bisulfite was dissolved in 250 ml of distilled water. Five tenths g of dry 1-amino-2-naphthol-4-sulfonic acid and 1.5 g of anhydrous sodium sulfite were added. The solution was made up to 500 ml, shaken thoroughly and stored in a brown bottle in a cold room at about 10°C.

The phosphorus content was calculated by the following formulas:

$$k_1 = \frac{100\ (2 - \log S1)}{0.2}$$

$$k_2 = \frac{100\ (2 - \log S2)}{0.4}$$

$$k_3 = \frac{100\ (2 - \log S3)}{0.6}$$

$$k_4 = \frac{100\ (2 - \log S4)}{0.8}$$

$$k_5 = \frac{100\ (2 - \log S5)}{1.0}$$

$$K = \frac{k_1 - k_2 - k_3 - k_4 - k_5}{5}$$

$$P = \frac{1000\ (2 - \log \text{of sample reading})}{K}$$

where S1, S2, S3, etc. are the colorimeter readings for the standards containing 0.2, 0.4, 0.6 mg etc. of phosphorus respectively. P equals the weight of phosphorus in mg.

Choline

An aliquot containing 0.2 to 0.7 g of fat was pipetted into a 125-ml Erlenmeyer flask and the solvent evaporated. The fat sample was saponified for two hours at 80°C. with 20 ml of saturated barium hydroxide. Then the samples was neutralized with glacial acetic acid using phenolphthalein as an indicator. The neutralized solution was filtered and the insoluble material washed several times with small portions of distilled water. Ten ml of 2 percent amonium reineckate in methanol were added. The samples were stoppered and allowed to remain at 10°C. for 12 hours or overnight to insure complete precipitation.

While still cold the insoluble reineckate-choline complex was filtered with suction unto an asbestos pad. The asbestos was supported by a Gooch button in the bottom of a short stemmed funnel. The precipitate was washed with cold 95 percent ethyl alcohol until the washings were colorless. Then the precipitate was dissolved with acetone and collected in Evelyn colorimeter tubes. The asbestos pad was washed with acetone until colorless. All samples were made up to a known volume with acetone and the color intensity measured on an Evelyn photoelectric colorimeter

between 30 and 80 percent transmission using a 515 filter. Standards were also prepared and run along with the unknowns.

A standard solution of choline was prepared by dissolving one g of choline in 100 ml of distilled water and taking a 10-ml aliquot and making up to a liter with water. The choline was weighed rapidly so it would not absorb atmospheric moisture. One ml of the standard solution contained 0.1 mg choline. The standard concentrations used were 0.1, 0.2, 0.4 and 0.6 mg of choline.

A factor, K, was determined from the standard readings:

$$K = \frac{\text{micrograms of choline}}{\text{volume solution} \times (2 - \log. \text{ galvanometer reading})}$$

The formula for calculating unknowns was :

Micrograms choline = K x volume soln. x (2 - log gal. reading)

Ethylene Chloride Extraction

The weighed tissue was cut into fine pieces and placed in a 500-ml distilling flask. Enough ethylene chloride was added to cover the tissue. The mouth of the flask was stoppered with a glass stopper and the side arm of the flask extended into a water condenser. The mixture was heated over a bunsen burner and the distillate collected in Erlenmeyer flasks. The tissue in the flask was kept covered by frequent additions of ethylene chloride. The solvent in the flask grew darker in color as heating continued. The heating was continued until the distillate was clear, indicating that all the water had been removed from the tissue.

The solvent was filtered hot through coarse filter paper into 250-ml volumetric flasks. The tissue was washed several times with hot ethylene chloride. When cooled, the extracted fat was made up to volume, the percentage of fat was calculated by determining the amount of fat in an aliquot of the solution. The distilled ethylene chloride could be reused by freezing and removal of ice by filtration.

Acid Value

An aliquot containing 0.5 g of fat was pipetted into a 125-ml Erlenmeyer flask. Then 5 ml of carefully neutralized alcohol was added and the solution heated to near boiling. The sample was titrated with N/100 alcoholic potassium hydroxide using a microburette and phenolphthalein as an indicator. The end point was taken when the solution retained a pink color for one minute.

The acid value of a fat was recorded as percentage of oleic acid :

$$\frac{\text{ml titration} \times \text{normality KOH} \times .282 \times 100}{\text{sample weight}}$$

Peroxide Value (2)

An aliquot containing 0.5 g of fat was pipetted into a 125-ml ground-glass flask and 10 ml of chloroform-acetic acid solution was added. Exactly 1 ml of a saturated solution of potassium iodide was added from a pipette, the

flask was tightly stoppered and shaken for exactly 1 minute. The stopper and sides of the flask were then washed with 5 ml of distilled water, a few drops of starch indicator added and the sample was titrated immediately with N/100 thiosulfate solution to a colorless end point.

Saturated potassium iodide-Thirty g of potassium iodide was added to 20 ml of distilled water and shaken. The saturated solution was kept in the dark as it turns yellow when exposed to the light. If the solution was yellow it was necessary to run a blank determination along with the samples.

Chloroform-acetic acid-One-hundred and fifty ml of chloroform was added to 300 ml of glacial acetic acid and the solution was mixed well.

N/100 thiosulfate solution-Two and forty eight hundreths of a g of sodium thiosulfate was dissolved in one liter of distilled, and the exact normality determined by the following procedure:

Exactly 2 ml of N/10 potassium dichromate was measured into an iodine flask, 1 ml of concentrated hydrochloric acid and 2 ml of 15 percent potassium iodide solution were added. The mixture was immediately titrated to a greenish color with thiosulfate solution. Starch indicator was then added. Titration was continued slowly with occasional

shaking until the solution suddenly turned a clear, bright green.

The following formulas were used in calculating the peroxide value:

$$\text{Normality thiosulfate} = \frac{\text{normality of dichromate}}{\text{ml of thiosulfate used}}$$

$$\text{Peroxide number} = \frac{\text{ml titration} \times \text{N thio.} \times 0.5 \times 1000}{\text{sample weight}}$$

Aldehyde Determination

An aliquot containing 0.5 g of fat was pipetted into a marked ignition tube. Two blanks were run along with the unknowns. The solvent was evaporated to about 5 ml, the amount being the same for each sample. Sufficient special Skellysolve B was added to make to a volume of 25 ml. To this solution was added exactly 5 ml of rosaniline reagent. Cork stoppers covered with cellophane were inserted into the ignition tubes and secured. The tubes were placed in a specially constructed wire basket and shaken rapidly and in rythmn for two minutes. They were then allowed to stand for five minutes. Approximately 15 ml of the upper solvent layer was transferred to a pyrex centrifuge tube and centrifuged for 15 minutes at 1500 rpm. Approximately 13 ml of the solvent layer was carefully transferred to an Evelyn colorimeter tube and the transmittancy read at 515 mu. The samples were read against a blank which was set at 100 percent transmission.

Rosaniline reagent-Twenty g of pure rosaniline hydrochloride was weighed and placed in a liter volumetric flask. The reagent was dissolved in approximately 600 ml of absolute alcohol by shaking vigorously and then made up to volume with alcohol. The solution was allowed to stand for several days and any dark sediment which may have formed was filtered off. Exactly 500 ml of this clear solution was transferred to a liter volumetric flask and 133 ml of a 0.1 molar aqueous solution of sulfur dioxide added. This solution was made by dissolving 6.4 g of sulfur dioxide in a liter of distilled water. The rosaniline reagent was made to volume with distilled water and stored in glass stoppered bottles in the refrigerator.

Fat solvent-Skellysolve B was purified by shaking with 125 ml concentrated sulfuric acid per liter of solvent. The acid was drained off and the washings repeated until the acid phase was only slightly yellow. The Skellysolve B was then washed with water several times. The solvent was refluxed with 50 ml of a 50 percent solution of sodium hydroxide for approximately two hours, decanted and distilled over 50 g of calcium oxide. The purified solvent was stored in the dark.

$$\text{Schibstead's aldehyde number} = \frac{\text{optical density} \times 10}{\text{g sample}}$$

$$\text{Optical density} = \log \frac{1}{\text{transmission}}$$

The aldehyde numbers were recorded in Conrad's units. One-hundred Schibstead units equal 270 Conrad's untis.

Organoleptic Procedures(17)

The turkey leg obtained from the frozen carcass was allowed to warm to room temperature. The unskinned legs were washed with cold water, cleaned and placed in casseroles. One cup of water was added and the samples were cooked, covered, in a preheated rotary oven at 350°F. for one and one half hours. The covered casseroles were removed from the oven and allowed to cool for 15 minutes. The palatability committee judged the odor of the cooked turkeys while hot and recorded the ratings. A scale for evaluations from 1 to 10 was used. These numerical values carried the ratings of:

10. extremely good
9. very good
8. good
7. medium, plus
6. medium
5. medium, minus
4. fair
3. poor
2. very poor
1. extremely poor

Descriptive adjectives suggested to the judges for evaluating aroma were:

flat	bitter
faint	foreign
mild	fishy
mellowed	oily
high, gamey	acid or sour
strong	sweet
sharp	pronounced
rich	putrid
raw	normal
old	abnormal
oxidized	

RESULTS

Two basal rations were used, Tables 1 and 2. Ration #1 was developed to eliminate the more unsaturated fatty acids from the diet. Spectrophotometric analysis of ration #1 indicated that no linolenic or arachidonic acids were present. Ration #2 was a Kansas State College basal poultry ration. It contained 3.7 percent of fat which had an iodine value of 116.4. The various fats used to supplement ration #1 were hydrogenated fat, linseed oil, and the extract of alfalfa leaf meal, Table 3. The percentage of fat and the iodine value of the extracts from these feeds are recorded in Table 4. The addition of linseed oil produced a ration with the highest iodine value and the ration containing hydrogenated fat had the lowest iodine value.

The average weight gains of each group at 8, 16 and 22 or 28 weeks are shown in Table 5. Turkeys raised on basal ration #1 showed more weight gain at 16 weeks than those raised on ration #2. There were no outstanding differences in the rate of growth, food consumption, or weight of birds caused by the dietary supplements to either basal ration. The turkeys on ration #2 were allowed to mature to 28 weeks and were therefore heavier in weight and contained more fat than those on ration #1.

The length of the induction periods was taken as a means of comparing stability of the fat extracted from the skins. They indicated that the addition of ethanolamine or choline to the diet produced more stable skin fat than unsupplemented groups, Table 5. The presence of saturated fat or carotenoids (in alfalfa leaf meal) increased the effectiveness of ethanolamine. Choline and ethanolamine were also effective when fed to turkeys kept on a typical turkey ration (#2), however, ethanolamine was more effective than choline.

Table 4 shows the percentage of fat extracted from the skins, gizzards and livers of birds in groups 1 to 9. The skin was highest in percentage of fat and the liver contained the least amount of fat. The percentage of phospholipid was greatest in the liver tissue. The addition of ethanolamine to the diet tended to lower the percentage of phospholipid deposited in the skin and gizzard. It was especially notable that on a diet containing one percent linseed oil, the gizzard and liver contained an almost equal percentage of phospholipid. However, when the diet which contained linseed oil was supplemented with ethanolamine or choline, the percentage of phospholipid fat in the gizzard dropped greatly, while there was a slight increase in the amount of phospholipid fat in the liver.

Turkeys on basal ration #2 deposited the highest percentage of total fat in the skin and the greatest per-

centage of phospholipids in the liver, Table 6. In the birds on this ration the effect of ethanolamine and choline on the amount of total and phospholipid fats was slight.

There was a significant difference in the iodine values obtained from the fats of the skin, gizzard and liver of groups 1 to 9, Table 4. This difference was caused by the varying degree of unsaturation of the dietary fat. There was no change in iodine value caused by feeding choline or ethanolamine. The iodine values obtained for fat extracted from turkeys in groups 10, 11 and 12, Table 6, showed no significant differences.

The results of spectrophotometric data, Table 7, seemed to explain how the feeding of small amounts of unsaturated fat or ethanolamine influenced the stability of the extracted fat. The fat extracted from the skin tissue of birds which had been supplemented with only 1 percent linseed oil, for example, contained 3.9 percent linolenic acid and 1.2 percent arachidonic acid. In comparison, the fat extracted from the skin tissue of birds which had not been supplemented with linseed oil contained only 0.1 percent linolenic and no arachidonic acid. Linolenic and arachidonic acids absorb oxygen more rapidly than oleic and linoleic acids and therefore would tend to produce a more unstable fat.

The feeding of ethanolamine tended to decrease the percentage of linolenic acid in the extracted fat of the skin. The percentage of arachidonic acid in the extracted fats of the skin, gizzard and liver was decreased when ethanolamine and choline were included in the diet. The fat of the liver contained no linolenic acid and a relatively large amount of arachidonic acid.

The supplementation of choline or ethanolamine to basal ration #2 produced no significant difference in the percentage of fatty acids in the skin, gizzard and liver, Table 6.

The acetone soluble and insoluble fractions, which contained the neutral fats and phospholipids respectively, were analyzed, Tables 8, 9 and 10 . The iodine values of the neutral fats were higher than those of the phospholipids Spectrophotometric analysis showed that there was almost no linolenic acid in the neutral triglycerides, however, linolenic acid was present in the phospholipids of the skin in the diet groups which did not receive ethanolamine. The percentage of arachidonic acid was higher in the phospholipid fraction than in the neutral fats of groups 1 to 9.

The spectrophotometric analysis of the neutral fats indicated that the addition of ethanolamine reduced the percentage of linoleic acid in the liver. Ethanolamine also seemed to change the ratio of oleic and saturated

acids in several groups; namely, groups 2 and 7 and groups 4 and 5 of the liver neutral fats and groups 4 and 5 of the gizzard neutral fats.

Analysis of the fatty acid composition of the acetone insoluble fraction showed that the addition of ethanolamine altered the ratio of oleic and saturated fatty acids in the liver of groups receiving one percent linseed oil. The addition of ethanolamine also reduced the percentage of linolenic acid in the phospholipid of the skin.

Tables 11 and 12 record the analysis of the acetone soluble and insoluble fractions of the skin, gizzard and liver of groups fed basal ration #2. These data indicate that the addition of ethanolamine produced little or no change in the fatty acid composition of the neutral fats. In the phospholipid fraction, ethanolamine and choline tended to reduce the percentage of linoleic and arachidonic acids. There was no linolenic acid deposited in the phospholipid fraction of the birds on ration #2.

The results of other experiments performed in this laboratory recently have indicated that certain fat-soluble compounds are able to interfere in a spectrophotometric analysis. The data from these experiments have shown that some of the contaminating compounds can be removed by saponification of the fats, discarding the non-saponifiable. Since the spectrophotometric analysis reported in the

present study was done on the triglycerides and not on the fatty acids, the present data of the fatty acid composition of the phospholipids do not present the most accurate information.

Chemical analysis showed that there was little difference in the phosphorus content among the turkey skins of the twelve groups, Table 13. Determinations for choline content in the skins were also performed. The results are recorded in Table 13, but these data were considered inaccurate and did not influence the conclusions of this work.

Ethanolamine and choline were administered in order to observe their action as fat stabilizers during cold storage. The effect of these supplements on poultry which had received the more unsaturated fats was of special interest.

The organoleptic tests indicated that after 4 months of storage all groups of turkeys were acceptable, Table 14. At 9 months, the groups which had received linseed oil and hydrogenated fat but no supplements of ethanolamine or choline were unacceptable. Similar groups which had been supplemented with ethanolamine were still acceptable. After 12 months storage only those birds in groups 10 and 11 (ration #2 control and a group receiving choline) were considered edible by the palatability committee. The

groups which received linseed oil, either alone or with ethanolamine or choline on basal ration #1, were considered to have the most pronounced oxidized flavors.

The characteristics of the extracted skin fat, Tables 15, 16 and 17, of the stored turkeys indicated that the birds which had received ethanolamine were more stable than the unsupplemented turkeys. In almost every case, the peroxide and aldehyde values of the fat extracted from the supplemented groups were lower than the values of the fat extracted from the unsupplemented groups. These differences were greatest after 4 and 9 months of storage in the groups which had been fed linseed oil.

Ethanolamine and choline were also effective when fed to birds kept on a typical turkey ration (basal ration #2), grain and grass range, similar to actual commerical practice. However, ethanolamine was more effective than choline.

Table 1. Basal ration #1.

Ingredients	Pounds
Skim milk powder	15.0
Dried brewers yeast	3.0
Corn gluten meal	12.0
Corn oil cake	12.0
Ground corn or sorghum	23.0
Gelatin	2.0
Soybean meal (solv. extract)	5.0
Ground oats	20.0
Limestone	2.0
Sodium chloride	0.9
Dicalcium phosphate	3.5
3.5 mg vitamin A conc.[1], 0.6 g tocopherol conc.[1], 1.6 mg crystalline vitamin D[2], 0.25 g folic acid [3], 0.06 g pyridoxin, 1 g niacin, 50 g choline, 10 g manganese sulfate.	

[1] Courtesy of Distillation Products, Rochester, N.Y.

[2] Courtesy of Du Pont Co., Wilmington, Delaware

[3] Courtesy of Lederle, Inc., Pearl River, N.Y.

Table 2. Basal ration #2.

Ingredients	Pounds
Yellow corn	20.0
Ground wheat	10.0
Ground oats	10.0
Wheat shorts	10.0
Wheat bran	5.0
Alfalfa leaf meal	7.0
Meat and bone scraps	20.0
Soybean meal	15.0
Ground limestone	2.0
Sodium chloride	1.0

150 g Prot-A [1], 50 g D-sec.[2], 3 g riboflavin mix, 15 g manganese sulfate, wheat, corn, and grass range ad libetum.

[1] A commerical vitamin A concentrate.

[2] A commerical vitamin D concentrate.

Table 3. Diet supplements.

Group	Supplement	Amount ethanol-amine per 100 pounds feed	Amount choline per 100 pounds feed
		To Basal Ration #1	
		grams	grams
1	1% linseed oil	---	---
2	10% alfalfa leaf meal	---	---
3	2% hydrogenated fat	---	---
4	none	---	---
5	none	100	---
6	2% hydrogenated fat	100	---
7	10% alfalfa leaf meal	100	---
8	1% linseed oil	100	---
9	1% linseed oil	100	50
		To Basal Ration #2	
10	none	---	---
11	none	---	50
12	none	100	---

Table 4. Percentage of total fat, percentage of phospholipids, and iodine values of groups 1 to 9

Characteristic	Supplement	Ration	Extracted tissue					
			Skin		Gizzard		Liver	
			None	Ethanolamine	None	Ethanolamone	None	Ethanolamine
Percentage fat extracted	none	2.8	22.6	26.3	4.3	5.8	2.7	3.2
	2% hydro. fat*	4.9	22.2	24.4	7.3	7.1	2.7	2.8
	1% linseed oil	3.6	26.2	29.2	3.9	5.4	3.3	2.9
	1% linseed oil	3.6	26.2	33.1**	3.9	8.2**	3.3	3.4**
	10% alfalfa	4.6	22.4	25.1	6.7	7.3	3.3	2.9
Percentage phospholipids extracted	none	--	4.1	4.1	16.8	9.2	42.2	26.5
	2% hydro. fat	--	4.5	3.6	8.8	7.7	31.1	42.2
	1% linseed oil	--	2.5	2.1	25.5	9.4	27.0	39.5
	1% linseed oil	--	2.5	2.3**	25.5	4.6**	27.0	27.6**
	10% alfalfa	--	4.2	2.1	7.7	6.9	32.7	35.4
Iodine value	none	113.4	87.7	88.4	88.2	84.8	84.4	80.7
	2% hydro. fat	86.7	83.3	80.9	83.9	80.0	99.3	88.9
	1% linseed oil	128.2	91.5	99.5	97.1	92.4	96.2	89.5
	1% linseed oil	128.2	91.5	97.9**	97.1	82.9**	96.2	88.7**
	10% alfalfa	103.6	87.9	89.8	85.8	85.7	94.5	91.2

* hydrogenated fat
** received 50 g/100 pounds ration of additional choline.

Table 5. Weight gain and induction period data.

Group	Supplement	Average weight of birds			Induction period	Relative effectiveness
		8	16 weeks	22	hours	
		pounds				
1	1% linseed oil	1.2	9.1	15.2	13	0.5
2	10% alfalfa leaf meal	1.3	7.7	12.8	17	0.6
3	2% hydrogenated fat	1.4	9.7	12.3	24	0.9
4	none	1.3	9.9	16.2	26	1.0
5	none + ethanolamine	1.3	8.8	14.8	94	3.6
6	2% hydro. fat + "	1.5	9.5	14.4	130	5.0
7	10% alfalfa + "	1.3	7.3	14.4	142	5.4
8	1% linseed oil + "	1.2	10.0	15.0	22	0.8
9	1% linseed oil + " + choline	1.5	8.9	14.1	29	1.1
10	none	1.6	7.1	17.8	19	1.0
11	" + choline	1.5	7.2	17.6	26	1.4
12	" + ethanolamine	1.6	7.3	17.3	39	2.0

Table 6. Composition of the fat extracted from birds kept on basal ration #2.

Tissue	Supplement	Percent fat	Percent phospho-lipids	Iodine value	Percentage of mixed fatty acids				
					Linoleic	Linolenic	Arachidonic	Oleic	Saturated
Skin	none	25.2	1.9	77.3	16.8	0.0	0.1	51.5	31.6
	choline	25.4	2.0	76.0	20.0	0.0	0.4	42.6	37.0
	ethanolamine	29.4	1.3	76.5	17.1	0.0	0.0	41.8	38.6
Gizzard	none	15.4	1.9	76.9	17.5	1.0	0.5	45.1	35.4
	choline	12.8	2.8	76.5	19.1	0.0	0.3	45.5	35.2
	ethanolamine	20.0	1.2	75.9	16.3	0.0	0.3	41.1	42.0
Liver	none	5.8	22.2	83.7	16.7	0.0	7.2	51.8	24.3
	choline	7.4	16.5	77.8	16.0	0.0	5.1	35.2	43.7
	ethanolamine	5.5	19.3	76.3	15.7	0.0	6.0	30.9	47.4

Table 7. Percentage of the mixed fatty acids of the fat extracted from birds kept on basal ration #1

Tissue	Supplement	Linoleic		Linolenic		Arachidonic		Oleic		Saturated	
		None	Eta.*	None	Eta.	None	Eta.	None	Eta.	None	Eta.
Skin	none	25.0	24.5	0.1	0.0	0.0	0.0	46.6	45.7	28.3	27.4
	hydro. fat	21.4	20.8	0.0	0.0	0.0	0.0	48.9	45.2	29.6	33.5
	linseed oil	26.5	28.1	3.9	1.9	1.2	1.5	36.8	42.4	31.6	26.1
	linseed oil	26.5	24.3**	3.9	4.4**	1.2	0.4**	36.8	44.8	31.6	26.0**
	alfalfa	20.1	26.0	2.0	0.0	0.0	0.0	50.9	47.3	26.9	26.7
Gizzard	none	30.4	24.8	0.0	0.0	2.2	1.8	26.2	35.5	46.4	35.9
	hydro. fat	15.1	19.3	0.0	0.0	1.3	0.5	57.8	47.9	25.7	32.2
	linseed oil	24.7	24.7	0.5	3.5	3.8	1.3	42.4	37.7	28.6	32.7
	linseed oil	24.7	25.3**	0.5	3.5**	3.8	0.8**	42.4	27.0	28.6	43.3**
	alfalfa	25.7	25.6	0.0	0.5	2.5	1.5	36.3	42.0	36.5	30.4
Liver	none	14.9	15.6	0.0	0.0	10.4	6.4	24.2	47.4	50.4	30.5
	hydro. fat	16.5	17.8	0.0	0.0	7.7	6.9	39.3	37.1	36.5	38.1
	linseed oil	20.6	13.2	0.0	0.0	9.8	8.5	28.8	41.2	40.7	37.1
	linseed oil	20.6	14.0**	0.0	0.0**	9.8	7.9**	28.8	41.2	40.7	36.9**
	alfalfa	16.3	17.9	0.0	0.0	8.9	7.4	41.1	37.7	33.6	36.9

* ethanolamine
** received 50 g/100 pounds ration of additional choline

Table 8. Analysis of acetone soluble and insoluble fractions

Group	Characteristic	Supplement	Skin		Gizzard		Liver	
			None	Ethanol-amine	None	Ethanol-amine	None	Ethanol-amine
4,5	% acetone soluble	none	95.9	95.9	83.2	90.8	57.8	73.5
	iodine value		92.9	94.8	93.7	92.0	116.9	97.0
	% acetone insol.		4.1	4.1	16.8	9.2	42.2	26.5
	iodine value		25.3	23.8	77.9	80.9	78.3	70.4
3,6	% acetone soluble	hydro. fat	95.5	96.4	91.3	92.3	68.9	57.8
	iodine value		87.6	87.6	89.5	86.6	111.9	103.9
	% acetone insol.		4.5	3.6	8.8	7.7	31.1	42.2
	iodine value		23.9	25.0	77.4	75.1	83.9	81.5
2,7	% acetone soluble	alfalfa leaf meal	95.9	97.9	92.4	93.0	67.3	63.6
	iodine value		93.2	95.2	93.2	93.8	114.8	100.4
	% acetone insol.		4.2	2.1	7.7	6.9	32.7	36.4
	iodine value		24.9	26.9	78.2	78.5	79.5	79.8
1,8	% acetone soluble	linseed oil	97.5	97.9	74.5	90.6	73.0	60.5
	iodine value		99.3	107.9	106.6	101.1	116.9	99.4
	% acetone insol.		2.5	2.1	25.5	9.4	27.0	39.5
	iodine value		25.8	33.0	72.7	82.8	109.7	80.8
1,9	% acetone soluble	linseed oil and choline	97.5	98.7	74.5	95.4	73.0	72.4
	iodine value		99.3	102.3	106.6	104.0	116.9	98.3
	% acetone sinsol.		2.5	2.3	25.5	4.6	27.0	27.6
	iodine value		25.8	25.2	72.7	87.4	109.7	82.4

Table 9. Fatty acid composition of acetone soluble fraction

Group	Characteristic : Percentage	Supplement	Skine		Gizzard		Liver	
			None	Ethanolamine	None	Ethanolamine	None	Ethanolamine
4,5	Linoleic acid	none	28.8	29.7	27.6	15.1	18.7	14.2
	Linolenic acid		0.0	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		0.0	0.7	0.6	0.0	6.0	4.8
	Oleic acid		44.6	42.9	46.9	71.8	69.9	61.4
	Saturated acids		26.4	26.7	24.9	13.1	5.4	19.6
3,6	Linoleic acid	hydrogenated fat	24.2	24.1	25.9	21.4	20.2	14.5
	Linolenic acid		0.0	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		0.0	0.0	0.4	0.3	6.8	4.6
	Oleic acid		48.7	45.7	45.8	51.6	58.6	69.0
	Saturated acids		27.1	29.9	27.9	26.9	14.5	11.9
2,7	Linoleic acid	alfalfa leaf meal	27.5	28.2	25.8	26.4	19.5	12.6
	Linolenic acid		0.0	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		1.0	0.5	0.6	0.0	4.3	5.3
	Oleic acid		45.2	46.9	49.4	50.9	73.4	59.4
	Saturated acids		26.6	24.5	24.2	22.7	2.8	20.5
1,8	Linoleic acid	linseed oil	30.5	32.8	30.2	26.9	19.6	12.6
	Linolenic acid		0.0	0.0	0.3	0.5	0.0	0.0
	Arachidonic acid		1.1	0.2	0.9	0.0	6.7	5.6
	Oleic acid		44.8	53.0	44.6	56.4	65.8	64.4
	Saturated acids		23.6	13.9	21.6	16.2	7.9	17.5
1,9	Linoleic acid	linseed oil and choline	30.5	29.6	30.2	25.1	19.6	11.0
	Linolenic acid		0.0	0.0	0.3	0.8	0.0	0.0
	Arachidonic acid		1.1	0.5	0.9	0.0	6.7	4.9
	Oleic acid		44.8	52.0	44.6	62.7	65.8	68.9
	Saturated acids		23.6	17.9	21.6	11.5	7.9	15.1

Table 10. Fatty acid composition of acetone insoluble fraction

Group	Characteristic Percentage	Supplement	Skin		Gizzard		Liver	
			None	Ethanolamine	None	Ethanolamine	None	Ethanolamine
4,5	Linoleic acid	none	18.9	18.5	12.8	14.9	15.4	17.3
	Linolenic acid		3.3	0.0	0.0	0.0	1.5	0.0
	Arachidonic acid		0.3	1.1	7.6	5.9	10.4	9.2
	Oleic acid		0.0	0.0	32.6	37.6	12.8	9.5
	Saturated acids		74.8	80.4	47.0	41.5	59.9	64.1
3,6	Linoleic acid	hydrogenated fat	16.6	19.2	15.3	10.3	20.9	17.9
	Linolenic acid		5.5	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		0.3	0.6	3.4	4.9	10.8	9.7
	Oleic acid		0.0	0.0	42.8	44.1	11.1	18.7
	Saturated acids		77.7	80.2	38.6	40.7	62.2	53.8
2,7	Linoleic acid	alfalfa leaf meal	17.9	11.9	14.1	13.2	19.8	18.8
	Linolenic acid		4.8	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		1.8	2.7	4.7	6.3	8.6	9.0
	Oleic acid		0.0	0.0	41.0	37.4	16.4	17.3
	Saturated acids		75.6	85.4	40.2	43.3	55.1	54.8
1,8	Linoleic acid	linseed oil	16.9	16.8	12.8	15.8	22.7	18.3
	Linolenic acid		2.2	0.0	0.0	0.0	0.0	0.0
	Arachidonic acid		0.6	2.9	6.8	7.1	9.9	9.5
	Oleic acid		0.0	0.0	29.7	33.6	39.7	17.6
	Saturated acids		80.2	80.3	50.7	43.5	17.8	54.6
1,9	Linoleic acid	linseed oil and choline	16.9	19.6	12.8	15.6	22.7	20.9
	Linolenic acid		2.2	2.7	0.0	0.0	0.0	0.0
	Arachidonic acid		0.6	0.0	6.8	7.3	9.9	9.9
	Oleic acid		0.0	0.0	29.7	38.6	39.7	12.5
	Saturated acids		80.2	77.7	50.7	38.5	17.8	56.6

Table 11. Analysis of acetone soluble fraction of fat extracted from turkeys on basal ration #2

Characteristic	Skin Groups			Gizzard Groups			Liver Groups		
	10	11	12	10	11	12	10	11	12
% acetone soluble	98.1	97.9	98.7	98.1	97.2	98.9	77.8	83.5	80.7
iodine value	79.8	79.1	78.5	79.1	80.5	76.5	96.3	80.0	84.5
% linoleic acid	18.2	20.9	18.1	21.6	23.1	17.9	17.1	10.9	15.9
% linolenic acid	0.1	0.3	0.6	0.0	0.0	0.8	0.0	0.9	0.0
% arachidonic acid	0.7	0.6	0.0	0.1	0.0	0.0	2.6	1.4	2.1
% oleic acid	49.2	41.7	48.9	43.8	42.9	46.1	62.8	59.1	54.1
% saturated acids	31.8	36.4	32.4	34.4	34.0	35.2	17.5	27.7	28.0

Table 12. Analysis of acetone insoluble fraction of fat extracted from turkeys on basal ration #2

Characteristic	Skin Groups			Gizzard Groups			Liver Groups		
	10	11	12	10	11	12	10	11	12
% acetone insoluble	1.9	2.0	1.3	1.9	2.8	1.2	22.2	16.5	19.3
iodine value	77.3	76.0	76.5	71.5	90.1	76.9	78.6	80.6	79.4
% linoleic acid	24.3	19.9	17.6	17.6	15.5	15.4	23.5	21.1	19.5
% linolenic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% arachidonic acid	0.4	1.3	0.0	10.9	8.5	7.5	12.2	12.4	9.8
% oleic acid	35.6	39.3	49.6	3.7	37.7	26.6	0.0	1.8	12.7
% saturated acids	39.8	39.5	32.9	67.8	38.5	50.5	48.6	64.7	78.1

Table 13. Phosphorus and choline content of the total skin fat of the various groups

Group	Percentage Phosphorus	Percentage Choline
1	0.31	0.004
2	0.36	0.003
3	0.32	0.014
4	0.41	0.002
5	0.30	0.039
6	0.34	0.001
7	0.32	0.027
8	0.26	0.002
9	0.44	0.003
10	0.31	0.002
11	0.34	0.004
12	0.31	0.003

Table 14. The effect of feeding ethanolamine on the organoleptic rating of stored turkeys

Group	Supplements to Basal Rations	4 months		9 months		12 months	
		None	Ethanol-amine	None	Ethanol-amine	None	Ethanol-amine
		To Basal Ration #1					
4,5	none	6.1	6.2	5.8	5.8	3.8	4.0
3,6	hydrogenated fat	5.6	5.1	3.7	4.1	4.2	3.8
2,7	alfalfa leaf meal	6.6	7.1	4.6	4.8	4.2	3.5
1,8	linseed oil	7.4	5.9	3.4	4.4	2.6	1.8
1,9	linseed oil and choline	--	6.9	--	5.4	--	3.0
		To Basal Ration #2					
10,12	none	8.0	8.1	6.9	7.2	3.0	5.3
11	choline(no ethanolamine)	7.1	--	5.4	--	5.8	--

Table 15. The effect of feeding ethanolamine on the acid value of fat extracted from the skin of stored turkeys

Group	Supplements to Basal Rations	4 months		9 months		12 months	
		None	Ethanol-amine	None	Ethanol-amine	None	Ethanol-amine
		To Basal Ration #1					
4,5	none	1.6	1.4	1.0	1.2	2.5	2.1
3,6	hydrogenated fat	2.5	1.4	5.7	2.1	3.3	4.2
2,7	alfalfa leaf meal	0.9	1.4	1.2	2.1	1.5	1.9
1,8	linseed oil	2.0	1.3	2.0	1.3	1.9	2.2
1,9	linseed oil and choline	--	1.2	--	2.2	--	2.6
		To Basal Ration #2					
10,12	none	0.6	0.5	1.1	0.8	1.1	1.3
11	choline (no ethanolamine)	0.8	--	0.7	--	0.8	--

Table 16. The effect of feeding ethanolamine on the aldehyde value of fat extracted from the skin of stored turkeys*

Group	Supplements to Basal Rations	4 months		9 months	
		None	Ethanol-amine	None	Ethanol-amine
		To Basal Ration #1			
4,5	none	7.2	8.9	28.2	29.6
3,6	hydrogenated fat	9.2	8.7	24.0	23.9
2,7	alfalfa leaf meal	10.3	10.1	46.2	31.5
1,8	linseed oil	9.6	8.3	50.2	40.7
1,9	linseed oil and choline	--	8.2	--	39.0
		To Basal Ration #2			
10,12	none	8.7	4.3	14.1	5.9
11	choline(no ethanol-amine)	8.9	--	13.9	--

* The aldehyde values after nine months of storage were not reported due to inconsistencies in the data.

Table 17. The effect of feeding ethanolamine on the peroxide value of the fat extracted from the skin of stored turkeys

Group	Supplements to Basal Rations	4 months		9 months		12 months	
		None	Ethanol-amine	None	Ethanol-amine	None	Ethanol-amine
		To Basal Ration #1					
4,5	none	4.8	3.9	31.1	27.7	42.0	29.5
3,6	hydrogenated fat	2.1	3.7	11.2	12.8	12.0	17.5
2,7	alfalfa leaf meal	12.5	15.8	22.5	18.5	37.9	23.1
1,8	linseed oil	31.1	14.1	42.6	31.8	46.5	47.6
1,9	linseed oil and choline	--	15.4	--	28.0	--	28.1
		To Basal Ration #2					
10,12	none	6.3	2.6	9.5	5.8	26.8	11.8
11	choline (no ethanolamine)	8.2	--	7.2	--	22.3	--

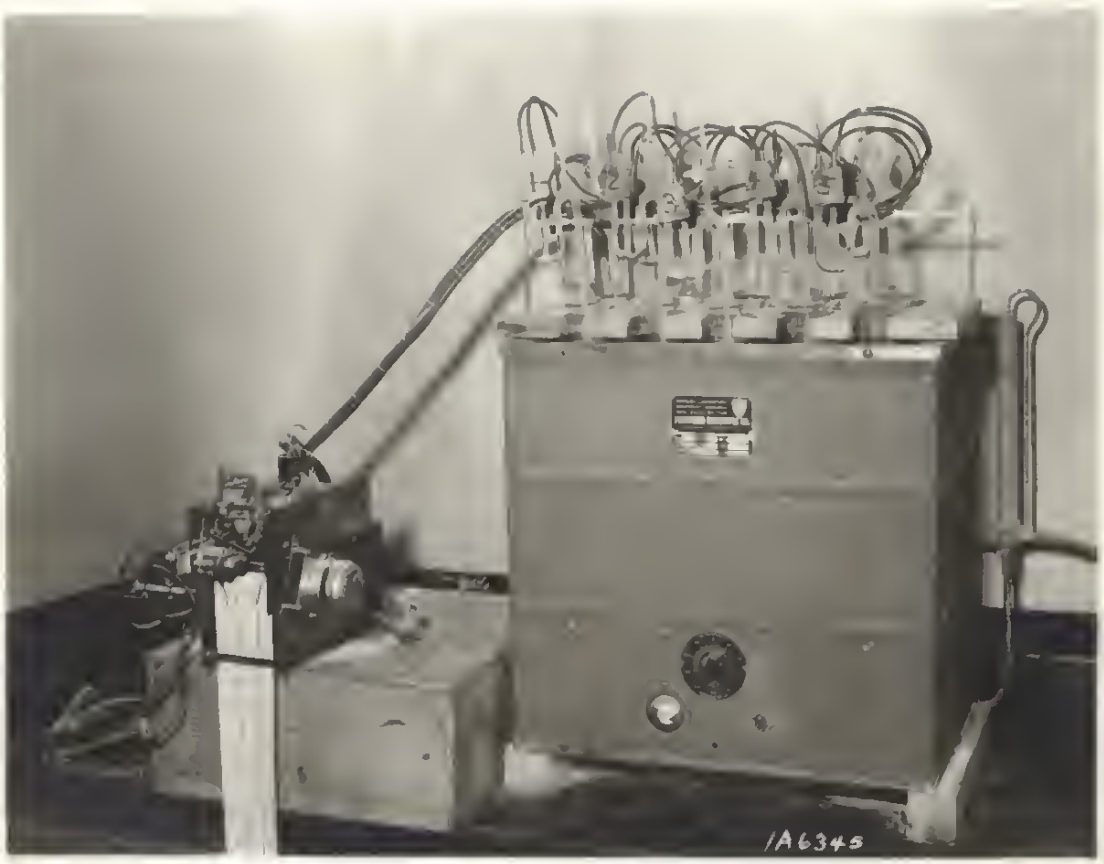

Fig.1. Induction period apparatus.

DISCUSSION

Barnes, et al. (1), Overman (15) and Kummerow, et al. (11) have recently made attempts to stabilize animal fats in vivo. Since the skin tissue is most readily exposed to oxidation it would seem that the stability of the turkeys could be improved by stimulating the deposition of saturated fats in the skin or increasing the amount of tissue stabilizer. The present data indicated that ethanolamine tended to remove the unsaturated fatty acids. Although the exact role of this supplement in fat metabolism was not determined, the results of the analysis suggested that ethanolamine may have functioned in two ways: (1) ethanolamine may have stimulated the catabolism of the unsaturated fatty acids thereby leaving the animal body with a higher percentage of saturated fatty acids or (2), ethanolamine may have caused the more unsaturated fatty acids to be synthesized into stable phospholipids.

The induction period data showed that the addition of ethanolamine to the diets of turkeys had a very definite stabilizing effect on the skin fats. Spectrophotometric analysis of the skin fats extracted from the turkeys showed that the addition of ethanolamine to the diet reduced the percentage of linolenic and arachidonic acids. Work recently carried out in this laboratory has substantiated the findings

of Holman and Elmer (10) that an increase in the number of double bonds of a fatty acid increases its susceptibility to oxidation. Pure fatty acid mixtures were combined in the same proportions as those found in the extracted fat of the skins of turkeys in groups 10, 11 and 12. Analysis of these artifical fatty-acid mixtures showed that when arachidonic acid was present the induction period was greatly shortened. Other pure fatty-acid combinations showed that the presence of linolenic acid also decreased the length of the induction period. These tests indicated that the amount of linolenic and arachidonic acids present could greatly alter the stability of fats to oxidation. Turkeys in basal ration #1 which received hydrogenated fat and alfalfa leaf meal supplemented with ethanolamine (groups 6 and 7) had the longest induction periods, or 130 and 142 hours, respectively. It is also notable that the skin fat extracted from these two groups contained no linolenic or arachidonic acids. These data indicate that ethanolamine affected the stability of turkey fats by influencing the distribution of the fatty acids in the various tissues.

The results of these tests also indicated that ethanolamine may have caused the more unsaturated fatty acids to be synthesized into phospholipids and in this form they would be more stable. This is indicated by the fact that lecithins have been shown to be fat stabilizers according

to the work of Wittka (23). Cephalin has also been proved as an antioxidant for purified fatty acids and esters by Olcott and Mattill (14). Since ethanolamine and choline are components of the phospholipids, lecithin and cephalin, the addition of these supplements to the turkey rations may have increased the amount of natural antioxidants present. The liver and gizzard tissues which contained the largest percentage of phospholipids, also contained a higher percentage of the more unsaturated fatty acids, especially arachidonic acid. This seemed to indicate that the unsaturated fatty acids may have been synthesized into phospholipids. Spectrophotometric analysis of the acetone-insoluble fractions also showed the liver and gizzard tissues contained a higher percentage of arachidonic acid in the phospholipid fraction. No conclusions could be drawn on the phospholipid analysis as the unsaponifiable fraction may have been contaminated with carotenoids which may have interfered with the analysis and altered the spectrophotometric readings sufficiently to produce errors.

In agreement with Hilditch (9), the iodine value does not indicate the fatty acid composition of a fat, and is therefore of little value in a study of fat metabolism. The iodine values of the extracted fats in this study were relatively constant and the use of spectrophotometric methods made a more complete fat analysis possible.

The complex chemical changes involved in the oxidation of fats during cold storage are not completely understood. According to Powick (16), the nature of these oxidative changes may vary from one substrate to another, particulatly under variable conditions of exposure to heat, air and light. Powick showed that rancidity was caused by the oxidation of fatty acids and that the glycerine and non-saponifiable fractions were not involved in this process. The presence of heptylic aldehyde has been suggested by this investigator to be responsible for the rancid odor of fats. However, the end product or products of oxidation of the fatty acids have not been acclaimed definitely. This fact allows for the difficulty in measuring rancidity by chemical means. White (22) found that the peroxide, Kreis and aldehyde tests were the most reliable chemical measures of rancidity. It is generally agreed that peroxide linkages are formed during the oxidation of fatty acids and therefore the peroxide test is a usual measure of rancidity. White has stated, "The precision of the aldehyde determination is poor, but it possesses the advantage of determining a portion of the material directly responsible for the rancid odour and flavour."

The data obtained in the study here reported seem to indicate that ethanolamine had a stabilizing effect on poultry skin fats during cold storage. The addition of ethanolamine to the poultry rations was especially bene-

ficial when the birds were not stored more than nine months. The presence of small amounts of highly unsaturated fatty acids seemed to bring out the stabilizing influence of ethanolamine.

Although a statistical correlation was not calculated for the results of the storage tests, there was general agreement regarding the changes in quality of the stored turkeys. The peroxide and aldehyde values increased during storage and the organoleptic ratings showed deterioration in flavor. It should be noted that any inconsistencies in this general trend may have been due to the variation between turkeys in the same group. According to Schreiber, et al.(17), "the fatter birds of a supposedly uniform group show greater fat oxidation during storage." The chemical tests employed in this study measure the oxidative changes which have occurred in the fats while organoleptic tests are an over-all measure of quality changes during storage. This fact may account for the variations in organoleptic ratings.

The results of this experiment point out the need for more basic knowledge of the metabolism of fats. More complete understanding of the phospholipid composition of the fats extracted from the various tissues is needed. Knowledge of fat metabolism could lead to controlled body fat composition and therefore to greater fat stability.

SUMMARY

The stabilization of frozen poultry against oxidative rancidity has been an economic problem receiving increased research interest within recent years. The purpose of this project was to study the effect of certain dietary variations on the stability of frozen turkeys and the composition of the fats extracted from the skin, gizzard and liver.

Two basal poultry rations were used. Ration #1 was developed to exclude the highly unsaturated fatty acids from the diet. The turkeys on ration #1 were divided into nine groups. Seven of these groups received fat increments. Two groups received 2 percent hydrogenated fat, two 10 percent alfalfa leaf meal and three 1 percent linseed oil. In each case one group was fed an additional supplement of ethanolamine. One of the groups which received one percent linseed oil received an additional supplement of choline. One group received only ethanolamine and another received no supplements whatsoever. Basal ration #2 was a standard poultry ration. There were three groups of turkeys on this ration; a control, one group receiving ethanolamine and another receiving choline.

Skin, gizzard and liver tissues of the fresh turkeys were extracted and the fats analyzed for induction period, iodine value, fatty acid composition, phospholipid per-

centage, phosphorus percentage and choline percentage. Five of the turkeys in each group were killed, dressed and stored at -13°C. One turkey in each group was analyzed after 4, 9 and 12 months of storage for peroxide value, aldehyde value, acid value and organoleptic rating.

The results of these tests indicated that ethanolamine, fed as a dietary supplement to growing turkeys, increased the stability of the carcass during storage. There was general agreement among the results of the peroxide, aldehyde and organoleptic tests. Choline was also found to stabilize poultry fats but not so much as ethanolamine. The results of the analysis of the extracted fats of the skin, gizzard and liver indicated that ethanolamine functioned by either saturating the body fats or metabolizing phospholipids. The exact role of this supplement in fat metabolism could not be determined. This work pointed out the need for more extensive knowledge of fat metabolism.

ACKNOWKEDGMENT

The author wishes to express her sincere appreciation to Dr. Gladys E. Vail, Head, Department of Foods and Nutrition, and to Dr. Fred A. Kummerow, Associate Professor in the Department of Chemistry, for their guidance throughout the course of research and also to those fellow students who assisted during the course of the experiment.

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